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Carbohydrate Research 337 (2002) 239–253

CARBOHYDRATE RESEARCH

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Coffee bean arabinogalactans: acidic polymers covalently linked to protein

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Received 8 October 2001; accepted 30 November 2001

Abstract

The arabinogalactan content of green coffee beans (*Coffea arabica* var. Yellow Caturra) was released by a combination of chemical extraction and enzymatic hydrolysis of the mannan–cellulose component of the wall. Several arabinogalactan fractions were isolated, purified by gel-permeation and ion-exchange chromatography and characterised by compositional and linkage analysis. The AG fractions contained between 6 and 8% glucuronic acid, and gave a positive test for the β-glucosyl-Yariv reagent, a stain specific for arabinogalactan-proteins. The protein component accounted for between 0.5 and 2.0% of the AGPs and contained between 7 and 12% hydroxyproline. The AG moieties displayed considerable heterogeneity with regard to their degree of arabinosylation and the extent and composition of their side-chains. They possessed a MW average of 650 kDa which ranged between 150 and 2000 kDa. An investigation of the structural features of the major AG fraction, released following enzymatic hydrolysis of the mannan–cellulose polymers, allowed a partial structure of coffee arabinogalactan to be proposed.

Keywords: Coffee; Arabica; Polysaccharides; Arabinogalactans; Arabinogalactan-proteins; Glucuronic acid

1. Introduction

Polysaccharides can exceed 50% of the dry weight of a green coffee bean.[1] Besides their sheer mass, a variety of evidences testifies to the important contribution that the polysaccharide content of the bean makes to the character of the final coffee brew.[2] Bean polysaccharide insolubility is a limiting factor for improved levels of coffee extract and polysaccharides also appear to play a role in sediment formation during the concentration of the coffee liquor.[3] It is therefore not surprising that studies to reveal the nature and properties of the polysaccharides in green and roasted coffee have been conducted for more than 40 years. It is known that in the major coffee species, Arabica and Robusta, (galacto)mannans, arabinogalactans, and cellulose constitute the predominant polysaccharides.[1] Although the polysaccharide composition of Arabica and Robusta beans is similar, there appear to be small but consistent differences in the relative amounts of galactomannan and arabinogalactan present in the two species.[4,5]

Linkage analysis of type II arabinogalactans has shown that they consist for the most part of $(1\rightarrow3)$-linked β-galactosyl residues, some of which are substituted at O-6 with arabinose and galactose residues.[6,7] A structure for coffee AG, which is consistent for these data, is that of the backbone of $(1\rightarrow3)$-galactosyl residues substituted at intervals in the O-6 position with various combinations of arabinosyl and galactosyl residues. In a recent study,[5] linkage data for arabinogalactans from green Arabica and Robusta beans supported this structural concept and demonstrated that AGs were heterogeneous, both with regard to the degree of branching in their backbone, and in the polymerisation of their side-chains.

During attempts to isolate and purify coffee AG in our laboratory, we observed that it is retained on anion-exchange matrixes. This suggested either the presence of an acidic sugar in the AG structure or that the AG was associated with a charged moiety, such as

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0008-6215/02/$ - see front matter
PII: S0008-6215(01)00316-0

protein or pectin. Glucuronic and galacturonic acids are known to be constituents of arabinogalactans from several sources[8] but as yet they have not been shown to be present in coffee arabinogalactans. Thaler[9] and Fischer et al.[5] liberated AG from cell walls of coffee beans using chlorodioxide treatment. It has been inferred from the Thaler result that the AG was covalently linked to structural protein in the cell wall,[10] but this has not been supported by subsequent research.

A major impediment to a more complete characterisation of the AG from the green bean is the insolubility of most of the polymer, even in strong alkali. Thus, AGs isolated from such extracts are not necessarily representative of the structure of the entire AG content of the bean. In the present study, we report on a procedure for the isolation and characterisation of AG from the cell wall material of Arabica beans which overcame this problem. In addition, we present data from several varieties of Robusta and Arabica beans which provide evidence that acidic arabinogalactan-proteins are a general feature of coffee cell walls.

2. Materials and methods

Plant material.—The beans were supplied as dried deparched coffee beans. The Arabica (*Coffea arabica*) varieties were Yellow Caturra, Catimor CIFC, and Sarchimor. The Robusta (*Coffea canephora*) varieties were Conillon, Ivory coast, and Indes. Yellow Caturra beans were subjected to a light, medium and dark roast as characterised by a colour test Neuhaus (CTN). The CTN values of 110, 80, 50 corresponded to a light (3–3.5 min), medium (4–5 min), and dark (7–8.5 min) roast at 240 °C.

Reagents and enzymes.—The β-glucosyl-Yariv reagent [1,3,5-tris(4-β-D-glucopyranosylphenylazo)-2,4,6-trihydroxybenzene] was purchased from Biosupplies Australia PTY, Ltd.

β-Mannanase (EC 3.2.1.78) from *Aspergillus niger*, cellulase II (*endo*-1,4-β-glucanase, EC 3.2.1.4), cellobiohydrolase II (EC 3.2.1.91) from *Trichoderma longibrachiatum* and α-L-arabinofuranosidase from *A. niger* (EC 3.2.1.55) were purchased from Megazyme International (Ireland). The *Humicola insolens* enzyme preparation, 'Ultraflo L', (Novo Nordisk, Denmark) is a multi-active β-glucanase preparation. The most important side activities are xylanase and arabinase. Before use, the enzyme was desalted on a PD-10 column (Pharmacia), freeze-dried and stored at 4 °C.

General procedures.—Polysaccharide fractions and CWM were analysed for their monosaccharide composition following hydrolysis in 72% H_2SO_4 for 3 h at rt and then in 1 M H_2SO_4 for 2 h at 110 °C. Monosaccharide analysis was carried out by GLC of the alditol acetates.[11] Uronic acid was determined quantitatively by the Blumenkrantz method.[12] Since this procedure does not discriminate between glucuronic and galacturonic acid, polysaccharide hydrolysates were also examined by HPAEC-PAD on a Dionex DX-500 using a CarboPac PA-1 column (4 × 250 mm) equilibrated in 150 mM NaOH. This gave an approximate value for the amounts of each uronic acid. TLC was done on HPTLC pre-coated plates (Silica Gel 60, Merck, Darmstadt, Germany) which were developed three times in 6:7:1 $CHCl_3$–HOAc–water. Compounds were visualised by the procedure of Hansen.[13]

Methylation of polysaccharides used a modification of the method of Ciucanu and Kerek.[14] GLC–MS of the partially methylated alditol acetates was accomplished using a SP-2380 column (30 m × 0.2 mm i.d., Supelco) maintained at 70 °C for 4 min, raised to 150 °C at 25 °C/min and then to 220 °C at 4 °C/min. The molar response factors reported by Sweet et al.[15] were used.

Carboxyl moieties of uronosyl residues in polysaccharides were reduced to the corresponding 6,6-dideuterioglycosyl residues prior to methylation using the procedure of Kim and Carpita.[16] For acidic oligosaccharides the carboxyl groups were converted to their methyl/esters with 0.5 M methanolic HCL (Supelco). Oligosaccharide (2 mg) was dissolved in 1.0 mL of the reagent and left at 30 °C for 2 h. *tert*-Butanol was added (0.5 mL), the solvent evaporated and the residue evaporated from MeOH three times. It was dissolved in 1 mL of D_2O containing 10 mg $NaBD_4$ and left at ambient temperature for 2 h. The excess borohydride was destroyed with 50 μL of AcOH and the reduced oligosaccharide evaporated several times from MeOH to remove borate. It was then desalted by passing through a bed of SP-Sephadex (H^+ form).

High-performance size-exclusion chromatography with multi-angle laser-light scattering detection (HPSEC-MALLS).—The measurements were carried out with HPSEC system (Bio-Tek Kontron) coupled to an Optilab-DSP differential refractive index detector and Dawn-DSP photometer (Wyatt Technology, USA). The column (30 cm × 7.8 mm (i.d.)) was a TSK-Gel PW_{XL} (TosoHaas) and the mobile phase consisted of 0.1 M NaCl at a flow rate of 1.0 mL/min. Arabinogalactan (10 mg/mL) was dissolved in 0.1 M NaCl. Prior to measurement, the instrument was calibrated and normalised using filtered HPLC quality toluene and a 48 000g/mol narrow pullulan standard in 0.1 M NaCl.

Electrospray mass spectrometry.—Native and methylated oligosaccharides were analysed using a Micromass (Manchester, UK) Quattro-LC triple quadrupole mass spectrometer equipped with a 'Z-Spray' electrospray ion source. The electrospray capillary voltage was set to 3.2 kV, the cone voltage to 60 V and the source block temperature to 90 °C. The cone gas was operated at 190 L/h, desolvation gas at 225 L/h and the desolvation temperature to 150 °C.

Data were acquired in positive mode from 50 to 1000 Th with a dwell time of 2 s. The samples were introduced at 10 μL/min in a mixture of 3:2 MeOH–water (pH 2.7 with AcOH).

Collision-induced dissociation spectra were acquired in continuum mode with argon as collision gas (3 mTorr), at a collision energy of 30 eV over a mass range of m/z 100 to m/z 600 Th.

Electron impact mass spectrometry.—The derivatized oligosaccharide was analysed after direct introduction into a mass spectrometer working in electron impact mode. The experiments were performed on a Finnigan MAT-8430 double focusing mass spectrometer (Bremen, Germany) working in electron impact at 70 eV with a source temperature set at 180 °C. The samples were dissolved in MeOH and heated at a rate of 2 °C/s. Mass spectra were acquired from 20 to 800 Da.

Isolation of AG fractions from Arabica Caturra.—*PAW-soluble.*—Arabica (var. Yellow Caturra) beans (60 g) were frozen in liquid nitrogen and cryo-milled to a fine powder. The powder was suspended in 300 mL of 2:1:1 phenol–AcOH–water (PAW) and stirred for 6 h at 4 °C and then overnight at ambient temperature. The supernatant was removed following centrifugation and the residue stirred for a further 5 h in 300 mL of fresh PAW. The PAW-soluble fractions were combined and dialysed. During dialysis, a precipitate formed which was filtered from the soluble fraction and discarded. The soluble fraction was concentrated to 200 mL and ammonium sulfate added to saturation (140 g) to precipitate proteins. The solution was left overnight and the precipitate which formed was discarded following centrifugation. The supernatant was dialysed for 4 days and freeze-dried (**AG 1**).

The PAW-insoluble residue was suspended in 300 mL of MeOH and stirred for 30 min. The supernatant was decanted and the residue re-extracted in 1:1 MeOH–$CHCl_3$ (500 mL) overnight. The residue (CWM) was finally suspended in water and dialysed for 3 days to remove all traces of PAW. The solution within the dialysis bag (**AG 2**) was separated from the CWM and both **AG 2** and the CWM were recovered by freeze-drying.

8 M KOH-soluble fractions.—CWM (1 g) was stirred for 4 h in 100 mL 8 M KOH containing 20 mM $NaBH_4$. The 8 M KOH-insoluble residue and 8 M KOH-soluble supernatant (**AG 3**) were separated by centrifugation and filtration (Whatman GFA glass fibre paper). Each fraction was adjusted to pH 5–6 with AcOH and dialysed for 3 days. During dialysis of the residue, additional polymers were solubilised and these were recovered as a separate fraction (**AG 4**).

Enzymatic solubilisation of AG from 8 M KOH-insoluble residue.—The 8 M KOH-insoluble residue (100 mg) was suspended in 5 mL of 0.05 M acetate buffer, pH 4.0, containing 0.02% sodium azide. Mannanase (30 U), cellulase II (15 U) and cellobiohydrolase (15 U) were added and the mixture stirred for 48 h at 37 °C. The supernatant was recovered, the pH adjusted to 5.5 and the solution heated at 100 °C for 10 min to inactivate the enzymes. The solution, containing the arabinogalactan was dialysed (MWCO 6–8 kDa) and freeze-dried (**AG 5**).

*Purification of **AG 1–5**.*—The polysaccharides in fractions **AG 1–5** each contained small amounts of mannan, galactomannan and/or pectic polysaccharides. Each was purified by a combination of gel-permeation and anion-exchange chromatography. A column of Sephacryl S-300 high resolution (2.5 × 100 cm) was equilibrated in 0.05 M imidazole–HCl buffer, pH 6.5. Samples (20–40 mg) were dissolved in the buffer (1 mL) and 5 mL fractions were collected and assayed for carbohydrate content by the phenol–H_2SO_4 method.[17]

A column (2.5 × 9 cm) of DEAE-Sepharose CL-6B was equilibrated in the same buffer used for gel-permeation chromatography. The samples were followed by 100 mL of 0.05 M buffer to remove all non-bound components. The retained fraction was then recovered from the column by elution with 150 mL of 1 M buffer.

An additional refinement was made for the further separation of **AG 5** into fractions **AG 5F1** and **AG 5F2**. A larger column of DEAE Sepharose CL-6B was used (2.5 × 18.5 cm) and elution with 1 M buffer was replaced by a linear gradient consisting of 250 mL of 0.05 M buffer and 250 mL of 1 M buffer. Fractions (10 mL) were collected and assayed for carbohydrate by the phenol–H_2SO_4 method. All polysaccharides were recovered following dialysis and freeze-drying.

Yariv assay for arabinogalactan proteins.—This was a modification of the protocol described by van Holst and Clarke.[18] AG fractions (4 mg) were dissolved in 1 mL of 0.15 M NaCl containing 0.02% sodium azide. Agarose gels (1%) containing the Yariv reagent (0.002%), 0.15 M NaCl and 0.02% sodium azide, were poured into Petri dishes to give a layer of agarose approximately 3 mm thick. Wells were made (4 mm in width) in the gel and 15 μL of a polysaccharide solution pipetted into the wells. The reagents without polysaccharide were used as blanks and gum arabic and larch arabinogalactan were used as test polysaccharides. The Petri dishes were sealed with parafilm and left in a dark cupboard at ambient temperature for 2 days to allow the coloured halo to develop.

*Partial-acid hydrolysis of **AG 5F1** with oxalic acid.*—**AG 5F1** from Arabica Caturra (60 mg) was dissolved in 6 mL of 12.5 mM oxalic acid and heated for 4 h at 100 °C. The hydrolysate was dialysed (MWCO 3.5 kDa) and the retentate (**Ox-AG 5F1**) recovered by freeze-drying.

*Enzymatic removal of arabinose from **AG 5F1**.*—The polymer (2 mg) was incubated with α-L-arabinofuranosidase from *A. niger* (1.76 U, Megazyme) in 0.3 mL of AcONa (67 mM, pH 4.0) at 35 °C for 17 h. The degraded polymer was recovered after dialysis and freeze-drying.

*Isolation of acidic trisaccharide from **AG 5F1**.*—**AG 5F1** (30 mg) was dissolved in 1:1:98 pyridine–AcOH–water (1 mL) containing 1.5 mg of 'Ultraflo L' enzyme preparation. The solution was incubated for 60 h at 32 °C. The hydrolysate was heated to 100 °C for 15 min to inactivate the enzymes. The slight precipitate was removed by centrifugation and the supernatant subjected to chromatography on a column of Toyopearl HW-40 S (100 × 1.6 cm). The fractions (3.5 mL) were assayed for uronic acid and the major peak subjected to anion-exchange chromatography on a column of QAE-A25 Sephadex equilibrated in 0.05 M pyridine–AcOH buffer. The retained material was recovered by eluting the column with a gradient of 100 mL of 0.05 M buffer and 100 mL of 1 M buffer and the major phenol–H_2SO_4 positive peak subjected to further purification by HPAEC on the Dionex.

*Smith degradation of **AG 5F1**.*—**AG 5F1** (10 mg) was dissolved in 50 mM $NaIO_4$ made up in 0.25 M formic acid (5 mL). The solution was adjusted to pH 3.7 with 1 M NaOH and incubated at 4 °C for 6 days with occasional stirring. Ethane-1,2-diol (1 mL) was added and the solution left for 1 h to destroy excess periodate. $NaBH_4$ (5 mL, 0.5 M) in 1 M NaOH was added and the solution incubated for 24 h at 25 °C. It was cooled to 0 °C and 1.5 mL AcOH added to destroy $NaBH_4$. The solution was desalted by dialysis, made up in 0.01 M with respect to TFA (pH 2.0) and heated at 100 °C for 10 min. The sample was examined by compositional analysis and gel-permeation chromatography.

Isolation of cell-wall material (CWM) from Arabica and Robusta beans.—Approximately 30 g of beans from each variety was frozen in liquid nitrogen and ground to a fine powder using a cryo-mill. The powders were freeze-dried overnight and 20 g of powder refluxed in 150 mL of 80% EtOH for 15 min. The supernatants were removed after centrifuging (6000 rpm) and 150 mL of fresh 80% EtOH added and the suspension stirred at rt for 2 h. The residue was recovered after centrifuging and extracted twice (2 h each time) in 1:1 MeOH–$CHCl_3$ and once in MeOH.

The residues were suspended in 150 mL of PAW (2:1:1 v/v) and stirred overnight at rt. The supernatants were removed and the residue re-extracted in 150 mL PAW for a further 3 h and then in 150 mL 70% MeOH for 2 h (twice). The residue was suspended in water and dialysed for 3 days. The supernatant from the dialysis bag was freeze-dried as a separate fraction, as was the residue (CWM).

3. Results and discussion

*Isolation of water- and alkali-soluble arabinogalactans (**AG 1–4**) from Arabica Yellow Caturra beans.*—Cell-wall material (CWM) was isolated from Arabica (Yellow Caturra) coffee beans by a method designed to minimise inadvertent degradation of the polysaccharides by either chemical or enzymatic breakdown, and maximise the removal of intracellular proteins which can co-purify with the CWM (Fig. 1). The PAW solubilised small amounts of polysaccharide (**AG 1**) and an additional fraction was solubilised during dialysis of the CWM (**AG 2**). The protein solubilised by PAW largely precipitated during dialysis. Additional protein was precipitated by the addition of ammonium sulfate to the PAW-soluble fraction.[19] The AGs remained in solution after this treatment. **AG 1** and **2** accounted for less than 5% of the total AG in the coffee bean. A more substantial AG fraction was solubilised by extracting the CWM in 8 M KOH (**AG 3**). As with the PAW extraction, an additional fraction was solubilised during dialysis of the 8 M KOH insoluble residue (**AG 4**). **AG 3** and **4** accounted for 32% of the total AG in the bean. The yield and monosaccharide composition of **AG 1**,**2**,**3** and **4**, the CWM and the 8 M KOH-insoluble residue are shown in Table 1.

*Isolation of cell wall-bound AG (**AG 5**) by enzymatic treatment.*—Approximately 60% of the coffee bean cell wall AG resisted extraction with 8 M KOH. Attempts

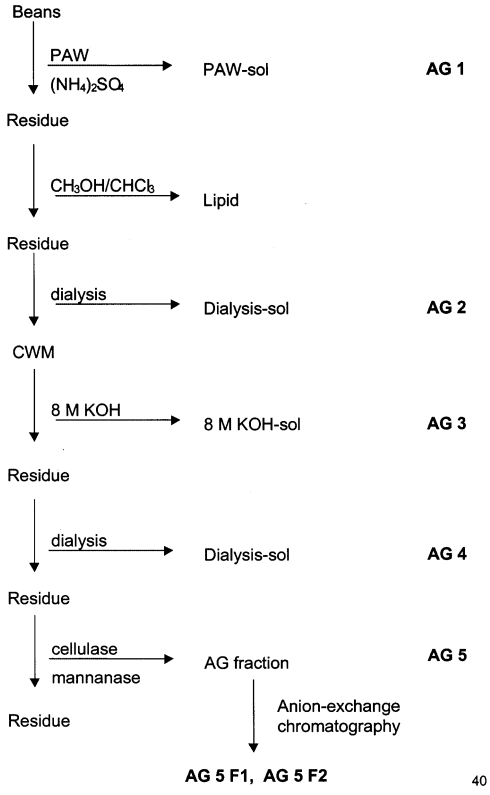


Fig. 1. Scheme for isolation of arabinogalactans from Arabica Caturra beans.

Table 1
Yield and composition of fractions isolated during preparation of the CWM and treatment of CWM with 8 M KOH

Fraction	Monosaccharide composition (mol%)							Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
Beans (mg/g)								
PAW-sol **AG 1**	4.5	1.5	0.8	34.6	0.7	6.8	52.6	3.0	383
Dialysis-sol **AG 2**	6.8	1.1	0.9	25.7	0.5	11.2	60.0	0.7	600
CWM	502	0.8	0.3	9.1	0.8	52.0	25.1	12.1	601
CWM (mg/g)								
8 M KOH-sol **AG 3**	86.0	1.1	1.0	19.6	4.1	29.0	43.6	1.7	626
Dialysis-sol **AG 4**	32.0	0.2	1.0	27.0	0.2	1.5	69.7	0.4	651
8 M KOH-residue	730	0.3	1.1	6.2	0.3	54.2	19.6	18.4	656

were therefore made to hydrolyse the cellulose and mannan content of the CWM with a mixture of mannanase, cellulase and cellobiohydrolase, thus releasing the AG held within the wall matrix. Initially, this was tried with CWM isolated after PAW extraction but a maximum of 40% of the CWM could be solubilised in this manner. However, enzyme treatment of the 8 M KOH-insoluble residue solubilised 93% of the residue. The procedure released 97% of the arabinose and galactose in the 8 M KOH residue in the form of **AG 5** (Table 2).

*Purification and linkage analysis of **AG 1–5**.*—**AG 1–5** were subjected to purification by gel-permeation and anion-exchange chromatography to remove contaminating polysaccharides. On Sephacryl S-300 the major AG peak in each fraction showed slight variations in their elution profiles but emerged near the void volume of the column, indicating a MW average of $\sim$ 500 kDa (Fig. 2).

AG 1 also contained a greater proportion of lower-molecular weight AGs than **AG 2**,**3**,**4** and **5**. Column fractions (marked in Fig. 2) were pooled and subjected to anion-exchange chromatography on DEAE Sepharose CL-6B. Neutral polymers were eluted from the column with 0.05 M buffer and the retained acidic polymers recovered in 1 M buffer and subjected to compositional and linkage analysis (Tables 3 and 4).

The most obvious trend in the monosaccharide composition of **AG 1–5** was a gradual increase in the Gal/Ara ratio in the fractions which were more difficult to extract. The Gal/Ara ratio ranged between 1.9 (**AG 1**) and 3.3 (**AG 5**), but some AGs in **AG 1** (Fb, Fig. 2) had a Gal/Ara ratio as low as 1.5. Nunes and Coimbra[20] reported that AGs extracted in hot water were more highly branched than those previously isolated from holocellulose. This reinforces the idea that some of the more highly branched AGs are more easily solubilised. **AG 5** eluted as the most compact peak during gel-permeation suggesting that it was the least polydispersed fraction.

Linkage analysis confirmed the highly branched nature of **AG1–5** (Table 4). In all fractions, the majority of the arabinosyl residues existed as nonreducing end-groups. A proportion of interchain 5-linked arabinosyl residues were also present and trace amounts of 2,5- and 3,5-linked arabinosyl residues indicated that approximately 10% of the 5-linked residues were themselves branched. The galactopyranosyl residues were mostly 3- and 3,6-linked, consistent with a galactan backbone of 3-linked galactose substituted at O-6 with arabinose/galactose sidechains. Structural differences between **AG 1** and **AG 5** were evident in two ways. **AG**

Table 2
Yield and neutral monosaccharide composition of **AG 5** and fractions resulting from treatment of 8 M KOH-insoluble residue with cellulase, cellobiohydrolase and mannanase

Fraction	Yield (mg/100 mg 8 M KOH-residue)	Monosaccharide composition (μg/mg)					
		Rha	Ara	Xyl	Man	Gal	Glc
AG 5	29.9	6.5	112.2		3.1	461.5	10.1
Low-molecular weight [a]	91.2		1.4	0.6	250.1	3.3	122.0
Residue	7.0	2.1	11.9	7.1	407.0	36.1	89.1

[a] Material not retained in 3.5 kDa cut-off dialysis membrane. Fraction contains in addition to low-molecular weight carbohydrates, amounts of salts contributed by the buffer, sodium azide and $(NH_4)_2SO_4$ from the enzyme preparations.

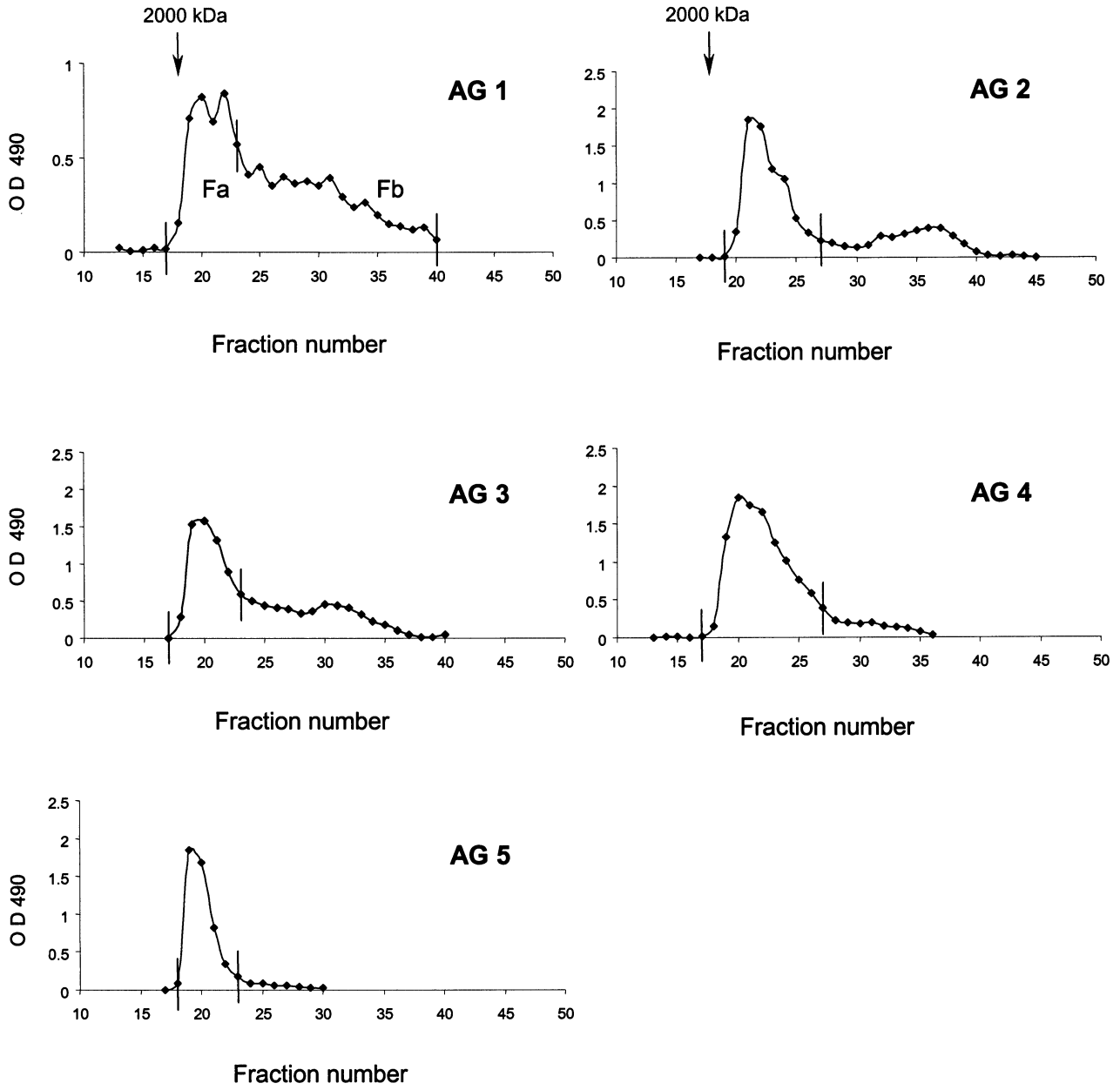


Fig. 2. Gel-permeation profiles on Sephacryl S-300 of fractions **AG 1–5**.

Table 3
Monosaccharide composition of purified fractions **AG 1–5**

Fraction	Monosaccharide composition (mol%)								Total (μg/mg)	Gal/Ara ratio
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid		
AG 1	1.9	1.0	30.5	0.4	0.4	58.1		7.8	750	1.9
AG 2	1.3	1.1	24.3	0.2	0.7	62.7	0.3	9.5	765	2.6
AG 3	1.4	1.1	25.2	0.7	0.2	61.0	0.4	10.1	801	2.4
AG 4	0.4	1.1	23.1			64.8		10.6	789	2.8
AG 5	0.4	1.2	21.0			69.4		7.9	753	3.3

Table 4
Glycosyl-linkage composition of Fractions **AG 1**, **3** and **5**

Sugar	Linkage	Linkage composition (mol%)		
		AG 1	**AG 3**	**AG 5**
Ara*f*	terminal	23.0	17.7	19.6
	5-	9.9	7.1	3.1
	2,5-	0.8	0.8	0.8
	3,5-	0.4	0.8	tc
Rha*p*	terminal	1.3	0.6	
Gal*p*	terminal	3.5	3.5	4.1
	3-	32.9	40.9	44.8
	4-	0.4	1.6	tc
	6-	1.8	1.6	2.5
	3,4-	0.9	1.2	0.8
	3,6-	25.1	24.1	24.2

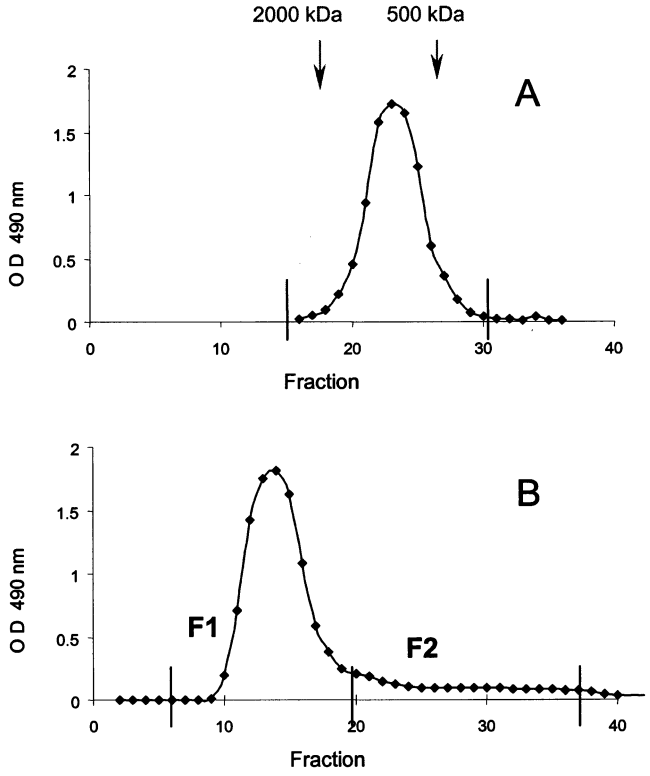


Fig. 3. Gel-permeation of **AG 5** following gel-permeation and anion-exchange chromatography. (A) Gel-permeation on Sephacryl S-400; (B) anion-exchange chromatography on DEAE CL-6B.

1 contained a threefold increase in the 5-linked interchain residues. Secondly, **AG 1** had an increased proportion of 3,6-linked galactosyl residues. These data are consistent with some of the increased arabinosyl content of **AG 1** being accommodated as additional 5-linked arabinosyl side chains on the galactan backbone.

Structural features of AG 5.—**AG 5** represented 60% of the arabinogalactan fraction in the bean. A more detailed structural characterisation of this polysaccharide was therefore undertaken.

Because **AG 5** eluted near the void volume of Sephacryl S-300 it was re-run on Sephacryl S-400 to verify the MW_{av} average of the polymer. It eluted earlier than dextran 500 indicating a $MW_{av} > 500$ kDa (Fig. 3(A)).

The uronic acid component in **AG 5** consisted of a mixture of glucuronic and galacturonic acids in the approximate ratio of 3:1 (data not shown). The additional presence of rhamnose suggested that some pectic polysaccharides may have co-purified with **AG 5**. **AG 5** was therefore subjected to anion-exchange chromatography using a buffer gradient. The elution profile (Fig. 3(B)) showed a single peak (**AG 5 F1**) followed by a long tail (**AG 5 F2**). The peak and tail were recovered as separate fractions and examined by compositional (Table 5) and linkage analysis (Table 6) before and after reduction of the carboxylic acid groups.

AG 5 F1 accounted for 80% of **AG 5** and **AG 5 F2** for the remaining 20%. Significantly, **AG 5 F2** contained three times the uronic acid content of **AG 5 F1** and most of the rhamnose. Linkage analysis of the carboxyl-reduced fraction revealed that all of the uronic acid in **AG 5 F1** existed as terminal glucuronosyl residues while **AG 5 F2** contained, in addition to terminal glucuronosyl residues, moderate amounts of 4-linked galacturonosyl residues and 2- and 2,4-linked rhamnose. These findings are consistent with the presence of a rhamnogalacturonan pectic polysaccharide in **AG 5 F2**. The fact that it was separated from the **AG 5 F1** by gradient elution is to be expected, since it is a more acidic molecule and would be retained more strongly on the exchanger.

Not all the uronic acid in the unreduced fractions could be accounted for as reduced moieties in the methylated samples. This is caused by incomplete reduction of the uronic acid by the carbodiimide reaction. Nevertheless, there was no trace of a second deuterated signal (m/z 207 for a terminal hexose derived from a uronic acid) in **AG 5 F1** other than that in the peak for terminal glucose. Terminal galactose in **AG 5 F1** contained no trace of m/z 207 and the only other deuterated signal was m/z 235 in **AG 5 F2** which was derived from the reduction of the $(1\rightarrow4)$-linked galacturonic acid. It was concluded from these data that glucuronic acid was the only uronic acid in **AG 5 F1** and was present solely as nonreducing end-groups.

The extreme chemical heterogeneity, with respect to the degree of branching or arabinosylation of coffee AGs, is emphasised by the composition of **AG 5 F1** and **AG 5 F2**. While **AG 5 F1** had a Gal/Ara ratio of 3:2, the latter had a Gal/Ara ratio of 2:1, similar to that of **AG 1**. Even within **AG 5 F1** there was chemical heterogeneity. A sample of **AG 5 F1** was subjected to gel-chromatography as in Fig. 3 and seven sub-fractions taken across the whole elution profile. Compositional

analysis (data not shown) revealed the Gal/Ara ratio varied from 3:2 and 2:4 between the beginning and end of the elution profile.

AG 5F1 was subjected to further molecular characterisation by HPSEC-MALL. The profile and values for the relevant parameters are shown in Fig. 4. The molecular weight determination by MALLS gave a MW of 654 kDa and a range between 150 and 2050 kDa, in approximate agreement with the values determined by gel-permeation chromatography (Fig. 3).

*Degradation of purified **AG 5F1** by partial-acid hydrolysis and arabinosidase treatment.*—A sample of **AG 5F1** was hydrolysed in 12.5 mM oxalic acid for 4 h at 100 °C. The hydrolysate was dialysed and the retentate containing the degraded polymer (**Ox-AG 5 F1**), was used for compositional (Table 7) and linkage analysis (Table 6).

The hydrolysis cleaved 90% of the arabinosyl residues which were released as monosaccharides. A dramatic increase in the proportion of terminal galactosyl residues and a less marked, but still significant, increase in the proportion of 6-linked galactosyl residues accompanied the loss of the terminal arabinosyl residues. This phenomenon was repeated when **AG 5 F1** was treated with arabinosidase which removed all but a trace of arabinose (Table 6). The increase in both terminal and 6-linked galactosyl residues following arabinosidase treatment indicates that in some cases the terminal arabinosyl residues are attached to the O-3 position of the galactose in a 6-linked galactosyl sidechain (their removal yielding a linear chain of 6-galactosyl residues). However, to account for the more marked increase in terminal galactosyl residues after arabinosidase treatment, most of the arabinosyl residues must terminate linear chains of either 3- or 6-linked galactosyl residues.

The glucuronosyl content of **Ox-AG 5 F1** was increased compared to the level in **AG 5 F1**, indicating that nearly all of the glucuronosyl residues were resistant to hydrolysis and were retained on the degraded polymer.

Following oxalic acid hydrolysis approximately 25% of the galactose content of **AG 5 F1** was recovered in

Table 5
Monosaccharide composition of **AG 5** fractions from DEAE Sepharose 6CL

Fraction	Monosaccharide composition (mol%)							Total (μg/mg)	
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid	
AG 5 F1	0.2	1.0	21.3	tc	tc	69.6	0.3	7.6	773
AG 5 F1 reduced		1.0	20.6	tc	tc	71.0	4.7	2.6	608
AG 5 F2	1.8	0.7	22.3	0.3	tc	48.4	0.5	25.9	707
AG 5 F2 reduced	2.7	1.0	23.4	0.3	tc	63.5	4.5	4.6	513

Table 6
Glycosyl-linkage composition of **AG 5 F1**, **AG 5 F2**, and **AG 5 F1** after partial-acid hydrolysis and arabinosidase treatment

Glycosyl residue	Linkage	Composition (mol%)					
		AG 5 F1		**AG 5 F2**		**Ox-AG 5F1**	Arabinosidase treated
		Original	Reduced	Original	Reduced	Reduced	Unreduced
Ara*f*	terminal	17.4	16.3	18.9	16.4		
	5-	2.9	4.6	6.6	7.6		
	2,5-			1.0	0.7		
	3,5-			1.6	1.2		
Rha*p*	2-			0.9	0.9		
	2,4-			1.0	1.4		
Gal*p*	terminal	3.0	3.9	3.6	3.9	19.7	23.4
	3-	44.4	43.0	38.4	32.9	42.2	42.4
	4-						
	6-	2.0	1.6	1.6	1.7	10.0	8.5
	3,4-	2.0	2.5	2.3	1.1	tc	1.2
	3,6	28.6	25.5	23.4	18.9	23.1	24.3
Gal A [a]	4-				10.7		
Glc A [a]	terminal		2.5		2.5	5.0	

[a] Analysed as 6,6-dideuterioglycosyl residues.

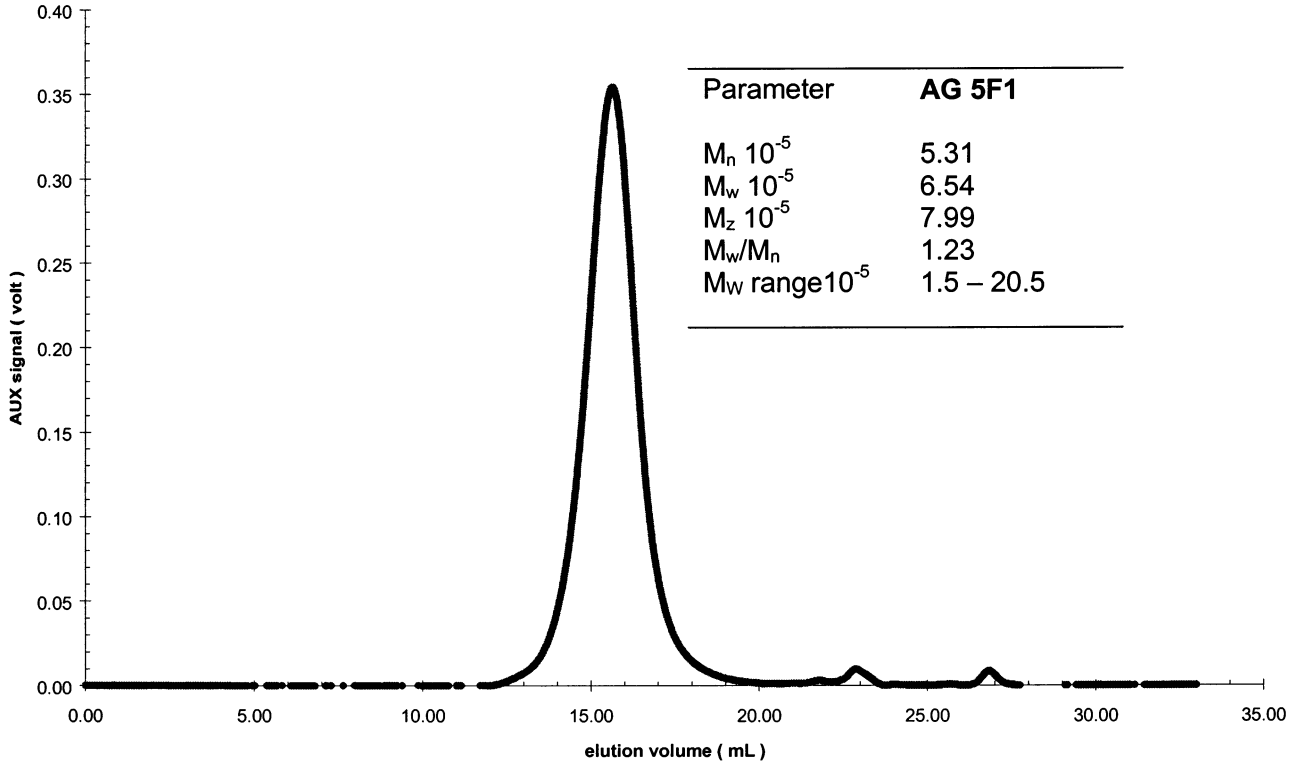


Fig. 4. HPSEC chromatogram of **AG 5F1** in 0.1 M NaCl at 25 °C detected by RI.

Table 7
Monosaccharide composition of **Ox-AF 5F1** before and after carboxyl-reduction

Fraction	Monosaccharide composition (mol%)								Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid	
Ox-AG 5 F1			2.4	0.1	tc	86.9		10.6	850
Ox-AG 5 F 1 reduced			2.7	1.0	tc	89.1	7.3		848

the low-molecular weight fraction which passed through the dialysis membrane. Of this fraction, 25% occurred as monosaccharide while the remainder was in the form of oligosaccharides. It is known that 6-linked hexopyranosyl residues are more stable than other linkage types to acid hydrolysis.[21] They are therefore unlikely to be cleaved by oxalic acid. This fact, allied to the finding that there was no marked increase in the proportion of 3-linked galactosyl residues in **Ox-AG 5F1** compared to **AG 5F1** suggests that few, if any, arabinosyl residues were attached directly to the backbone and that the initial linkage sugar unit in the side chains is a galactosyl residue.

Gel-permeation chromatography of **Ox-AG F1** on Sephacryl S-300 yielded a single uniform peak which possessed a MW_{av} average of < 10 kDa (Fig. 5). Since the original MW_{av} average of **AG 5 F1** was 650 kDa, this represented an extensive depolymerisation by hydrolytic conditions, designed to cleave mostly furanosidic linkages and this has previously been reported as evidence for the presence of arabinose in the AG backbone.[22] However, although glycosidic residues involving furanosides are hydrolysed faster than those of pyranosides by a factor of $10–10^3$,[21] a few pyranosidic residues will also be cleaved. If only five such glycosidic linkages in the backbone are hydrolysed, it could represent a 30-fold reduction in molecular weight. The low-molecular weight fraction released by the partial hydrolysis was separated by gel-permeation into a number of oligomeric fractions which were subjected to linkage analysis (data not shown). The oligomers possessed DPs between 2–6 and some contained high proportions of 3-linked galactosyl residues suggesting they were derived from fission of the AG backbone.

Periodate oxidation of **AG 5 F1** followed by a Smith degradation did not give a dramatic reduction in the

molecular weight of the molecule (Fig. 5), despite the fact that 90% of the arabinosyl residues were destroyed by the oxidation. This indicated that periodate vulnerable residues (i.e., 5-linked arabinosyl or 6-linked galactosyl) were not present in the AG backbone. There was only a slight loss of galactose residues from **AF 5 F1** during periodate oxidation; evidence that almost all the galactosyl residues are 3-linked in some form.

*Characterisation of acidic trisaccharide from **AG 5 F1**.*—Following the incubation of **AG 5 F1** with the enzyme preparation 'Ultraflo L', an acidic oligosaccharide fraction was purified from the enzymatic hydrolysate by a combination of gel-permeation and anion-exchange chromatography and HPLC on the Dionex (Fig. 6(A–C)). TLC (not shown) confirmed the presence of one major and one minor component oligosaccharide after anion-exchange chromatography which accounted for 90% of the total uronic acid content of **AG 5 F1**. The major oligosaccharide (larger peak Fig. 6(C)) was characterised by a combination of compositional and linkage analysis, mass spectrometry and TLC monitored degradation of the oligosaccharide with β-glucuronidase.

The oligosaccharide was converted to the alditol by borohydride reduction and then the carboxyl moiety of the uronic acid component reduced by borodeuteride treatment of the methyl ester. The carboxyl-reduced alditol was hydrolysed in TFA and yielded glucose and galactose in the ratio of 1:2. Linkage analysis indicated the presence of deuterated terminal Glc*p*, 6-Gal*p* and 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl galactitol (alditol from the reducing-end unit) in a ratio of 1.5:1.4:1. The lower than expected proportion of the reducing-end unit was attributed to some degradation of the reducing-end group during isolation and derivatisation of the oligosaccharide.

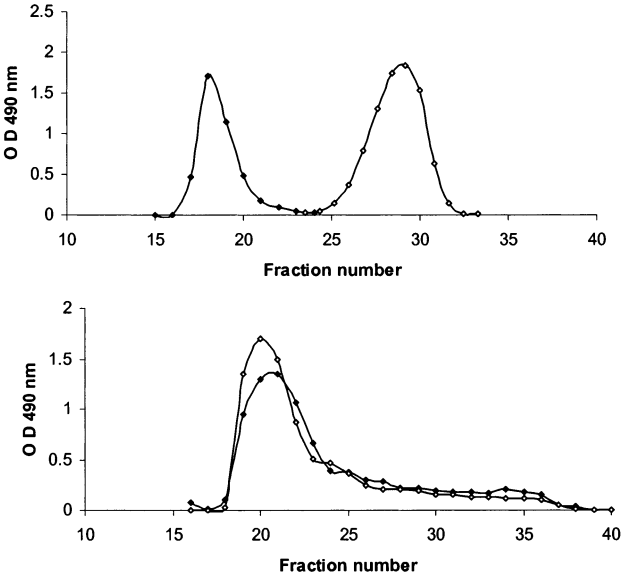


Fig. 5. Gel-permeation profiles of **AG 5F1** following partial-acid hydrolysis (upper profile) and Smith degradation (lower profile). ●, before and ○, after the respective treatments.

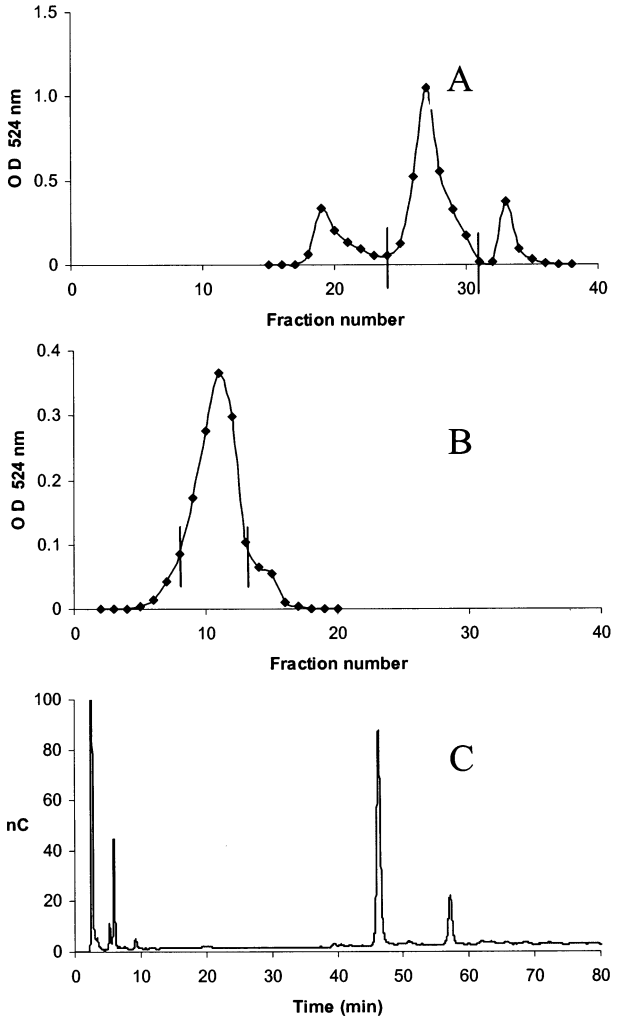


Fig. 6. Chromatography profiles at different stages of purification of acidic trisaccharide from enzymatic digest of **AG 5F1**. (A) Gel-permeation chromatography on Toyopearl HS-W40; (B) anion-exchange chromatography on QAE-Sephadex; (C) HPAEC-PAD chromatography.

The molecular weight of the underivatised oligosaccharide was determined at 518 Da by electrospray mass spectrometry using the two adduct ions at m/z 541 ($[M + Na]^+$) and 563 ($[M - H + 2\ Na]^+$). This mass corresponds to the molecular weight of a trisaccharide containing one hexuronic acid and two hexose residues. The minor component had a molecular weight of 680 Da, corresponding to a tetrasaccharide consisting of a single hexuronic acid and three hexose residues. Collision-induced dissociation of the ion at m/z 541 of the trisaccharide led to a product ion at m/z 365 confirming the presence of one hexuronic acid residue.

Electrospray mass spectrometry of the carboxyl-reduced methylated oligosaccharide detected an ion at

m/z 700 from the adduct ion $[M + Na]^+$ indicating a molecular weight of 677 Da for a methylated trisaccharide consisting of three hexose residues. Electron impact-mass spectrometry gave fragment ions at m/z 631, 543, 441, 440 and 236 formed after fragmentation of the methylated alditol derived from the reducing-end group. These allowed an unambiguous assignment of the structure as Glc-1,6-Gal-1,6-Gal.

Treatment of the trisaccharide with β-glucuronidase was monitored by TLC. The enzyme treated trisaccharide yielded glucuronic acid and a compound which ran at an R_f consistent with a Gal–Gal disaccharide, confirming the β configuration of the glucuronic acid residue.

These data lead to the conclusion that the acidic oligosaccharide was:

β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-D-Gal*p*

Coffee arabinogalactans as arabinogalactan-proteins (AGPs).—Many type II AGs are associated with proteins although concrete proof of the point of covalent attachment of the AG to the protein has been shown in very few cases.[23,24] These AGPs have been shown to specifically bind β-glycosyl-Yariv reagent, a red coloured dye which has been used to precipitate and purify AGPs.[25] Fig. 7 demonstrates the reaction of **AG 1**–**5** with the Yariv reagent using the single radial gel-diffusion method.[18] The Yariv reagent gave a strong positive reaction for all AG fractions but gave a negative reaction with Sigma Larch AG which lacks protein.

Amino acid composition of protein component.—The protein content calculated from amino acid analysis for **AG 1**, **3** and **5** was 1.9, 0.4 and 1.1%, respectively. As for other AGPs, the proteins were typically rich in hydroxyproline, serine, and alanine (Table 8).

Serine and hydroxyproline have been shown to be glycosylated in arabinose and galactose containing proteoglycans and glycoproteins. Fig. 7 demonstrated that even the AG fractions isolated after 8 M KOH treatment still gave a strong reaction to the Yariv reagent. Tentative identification of the amino acid involved in the glycosidic linkage can be made on the basis of its stability to alkali.[8] The glycosyl-serine or -threonine linkage is quite labile in mild alkali whereas glycosidic linkages to hydroxyproline are stable in even strong alkali. On this basis, it appears that in coffee AGPs the hydroxyproline-glycosidic linkage is present.

Arabinogalactan-proteins in different varieties of Robusta and Arabica beans.—Three varieties of Arabica and three varieties of Robusta beans were subjected to the same extraction protocol for AG isolation as described for the bulk Arabica Caturra sample. The monosaccharide composition for the total polysaccharide content of each variety is given in Table 9.

The polysaccharides of Robusta beans all contained relatively more AG than the Arabica, irrespective of differences in the absolute polysaccharide content. Although the relative differences in the total polysaccharide content for each variety (% dry weight) are accurate, the absolute values are probably on the low

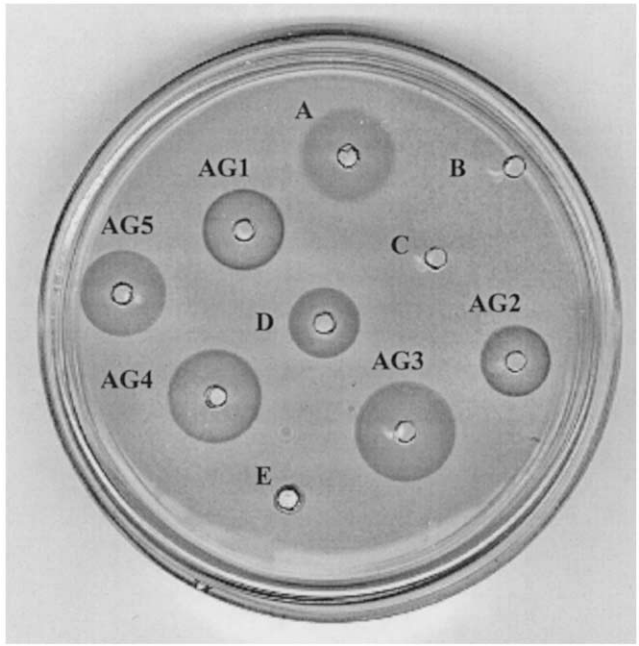


Fig. 7. Yariv gel-diffusion assay for arabinogalactan proteins **AG 1**–**5** are as defined in materials and methods. Samples A–E are: A, gum arabic; B, buffer, 0.01 M NaCl; C, Sigma Larch AG; D, AG isolated after enzyme treatment of non-alkali treated CWM; E, ammonium sulfate precipitate from PAW-soluble fractions.

Table 8
Amino acid composition (mol%) of arabinogalactan-proteins in fractions **AG 1**, **AG 3** and **AG 5F1**

Amino acid	**AG 1**	**AG 3**	**AG 5 F1**
Aspartic	8.7	8.0	7.2
Glutamic	10.3	11.2	10.1
Hydroxyproline	12.6	9.0	7.0
Serine	11.7	14.6	14.9
Glycine	8.3	11.4	11.4
Histidine	0.3	0.3	0.8
Arginine	1.3	2.4	0.7
Threonine	3.0	1.0	2.4
Alanine	17.9	11.6	12.2
Proline	5.8	4.4	5.8
Tyrosine	1.6	5.4	5.9
Valine	6.2	6.6	8.3
Methionine		1.6	1.4
Isoleucine	2.6	2.3	2.6
Leucine	5.4	5.8	5.0
Phenylalanine	1.1	1.7	2.4
Lysine	3.1	2.6	3.2
Total (% d.wt fraction)	1.9	0.4	1.1

Table 9
Monosaccharide composition of total polysaccharide in Robusta and Arabica varieties

Variety	Monosaccharide composition (mol%)								Total (% d.wt)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid [a]	
Robusta									
Conillon	0.8	0.3	10.7	0.7	42.1	26.4	15.0	3.9	50.6
Cote d'Ivoire	0.9	0.3	9.5	0.4	42.0	27.5	14.4	5.0	45.4
Indes	0.9	0.3	9.5	0.4	42.0	27.5	14.4	5.0	42.9
Arabica									
Catimor	0.6	0.3	8.8	0.6	47.0	23.7	14.7	4.4	45.7
Sarchimor	0.5	0.3	8.2	0.5	48.2	23.2	14.8	4.2	46.0
Yellow Caturra	0.6	0.3	8.2	0.6	47.2	23.0	15.3	4.9	45.0

[a] Approximately two thirds of the uronic acid was galacturonic acid and one third glucuronic acid.

Table 10
Monosaccharide composition of **AG 5** fractions in Robusta and Arabica varieties

Variety	Monosaccharide composition (mol%)								Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acid [a]	
Robusta									
Conillon	0.5	1.0	22.7	0.3	0.8	65.0	0.5	9.2	724
Cote d'Ivoire	0.4	1.0	22.0	0.3	0.5	66.5	0.3	9.1	747
Indes	0.6	1.0	19.8	0.2	0.6	65.8	0.3	11.7	700
Arabica									
Catimor	0.4	1.1	21.9	0.2	0.5	64.2	0.3	11.4	716
Sarchimor	0.4	1.0	21.1	0.3	0.6	65.5	0.3	10.7	700
Yellow Caturra	0.5	1.0	21.5	0.3	0.6	65.2	0.4	10.6	720

[a] Approximately two thirds of the uronic acid content was glucuronic acid and one third galacturonic acid.

side as they were obtained by summing the polysaccharide content of several fractions each of which had been processed through many extraction stages, so that some losses were inevitable.

AG 1–5 from all varieties showed a similar trend with **AG 1** being the most highly arabinosylated and **AG 5** the least. For Arabica Caturra, Catimor and Sarchimor, the Gal/Ara ratios ranged from 0.9 to 3.1, 1.5 to 3.2, 1.2 to 3.0, respectively. For Robusta Indes, Conillon and Ivoire the values were 0.9–3.1, 1.1–3.0 and 0.9–3.1, respectively.

The monosaccharide composition of **AG 5**, for each variety is shown in Table 10. There was no significant difference in the monosaccharide composition of **AG 5** between Robusta and Arabica. The uronic acid levels were determined by the colourimetric method of Blumenkrantz and so did not discriminate between glucuronic and galacturonic acid. However, HPLC of hydrolysates on the Dionex showed the presence of both glucuronic and galacturonic in the approximate proportions of 2:1 in all fractions.

Each **AG 5** fraction gave a strong reaction with the Yariv reagent (Fig. 8), indicating that arabinogalactan-proteins were a feature of both Robusta and Arabica varieties.

Arabinogalactan-proteins in roasted coffee.—AGPs were isolated from CWMs which had been prepared from Yellow Caturra beans at three roasting stages. They were separated by anion-exchange chromatography into F1 and F2 Fractions as described previously for **AG 5** and each subjected to the Yariv gel-diffusion assay. Both F1 and F2 Fractions gave a positive Yariv test at each roasting stage. Thus the protein-polysaccharide linkage of some of the AGPs remained intact even though extensive degradation of the polysaccharide moiety occurred at the longest roasting time.

4. Conclusion

The AGs of the coffee bean exist as an extremely heterogeneous mixture of arabinogalactan-proteins,

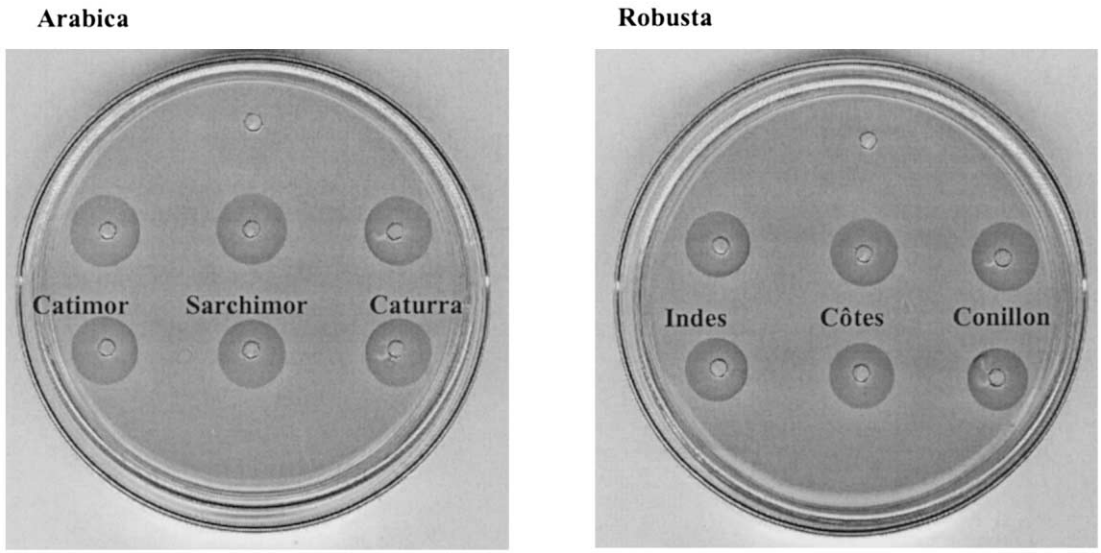
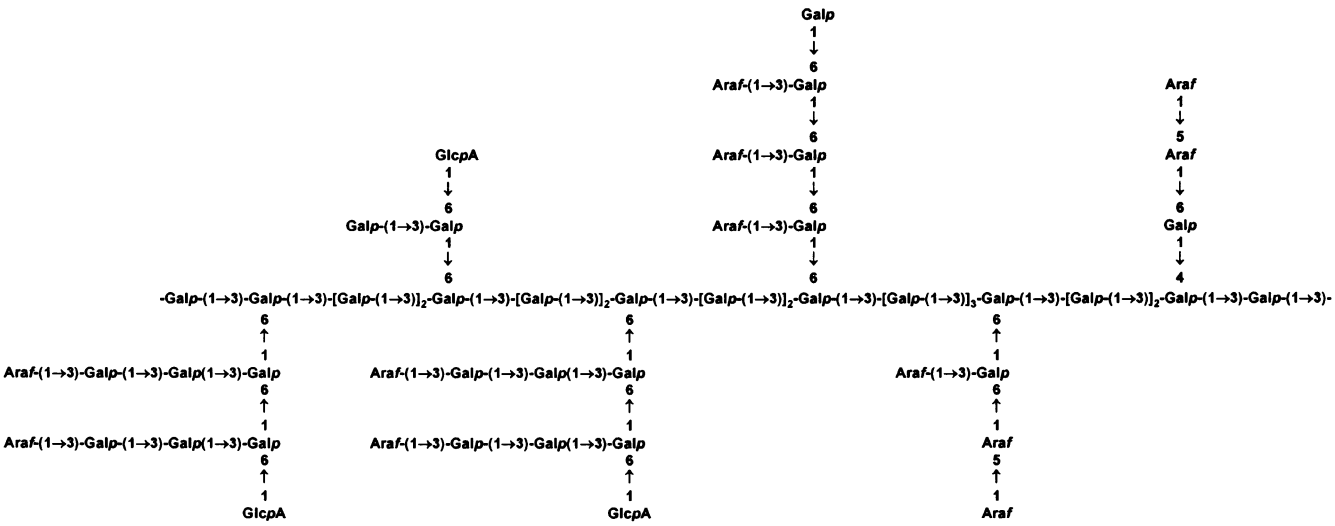


Fig. 8. Yariv gel-diffusion assay for arabinogalactan-proteins in **AG 5** fractions of Robusta and Arabica varieties. Top well contains Sigma Larch AG.

containing between 6 and 10% glucuronic acid and possessing a M_w average of ~ 650 kDa. Their heterogeneity relates particularly to their degree of branching and monosaccharide composition of their side chains. In those AG polymers which possessed a Gal/Ara ratio of ~ 3, as in **AG 5 F1**, $< 14\%$ of the arabinose occurred as 5-linked residues, the remainder existing as non-reducing terminal residues. A structure (one of many possible) for a partial sequence of **AG 5 F1** that is consistent with the combined results of linkage analysis of the intact and partially degraded **AG 5 F1** and the structure of the acidic trisaccharide, is shown in Scheme 1. The structure would not apply to those AGs in which the Gal/Ara ratio was ~ 2 or less. In these polymers, $> 30\%$ of the arabinose occurred as 5-linked arabinosyl residues, some of which are themselves branched, and a proposed structure would need to accommodate these additional features. The variation in the structural features of coffee AGs affect not only the physicochemical properties of the polymers, but potentially has implications for the structural integrity of the bean if it is found that the different forms are spatially separated. Just how the different structural forms of AG are distributed within the cell wall is not known, but we are currently using immunological and chemical probes to study cell-wall architecture in relation to AG distribution in the coffee bean.

For the first time, over 90% of the AG content of the coffee bean was solubilised by a combination of chemical and enzymatic treatments of the CWM. Over 60%



Scheme 1. One of many possible structures for the sub-unit of the carbohydrate moiety in **AG 5 F1**.

was released upon treatment of the alkali extracted CWM with a mixture of cellulose and mannan degrading enzymes. It seems likely that during suspension of the CWM in 8 M KOH, the cellulose and mannan structure of the cell wall is induced to swell, making the β-(1→4)-linked glycan chains more susceptible to hydrolysis. There was no evidence that the isolated AGs had been inadvertently modified or degraded by either 8 M KOH or the enzyme preparations used for their isolation. Sigma Larch AG showed an identical monosaccharide composition before and after treatment with either 8 M KOH or the enzyme cocktail used to isolate **AG 5**.

The existence of a covalent linkage between the AG polysaccharide and protein in coffee arabinogalactans was premised on: (1) The continued association of carbohydrate and protein during physical and chemical procedures of purification; (2) the positive reaction to the β-glycosyl-Yariv reagent; (3) the amino acid composition of the protein moiety which was hydroxyproline rich and characteristic of many AGPs previously reported. The protein component was low (0.4–1.9%) compared to the levels reported for many other AGPs and varied among the different AG fractions, as did the hydroxyproline content. Whether the variations represent different structural forms among the AGP fractions or merely reflect different degrees of co-purification of small amounts of intra-cellular non-AGP proteins during the isolation procedure is not known.

Based on the molecular weight of **AG 5F1** (650 kDa), and its protein content (1%), we can theorise about its possible structure. In a molecule of 650 kDa, the protein moiety might be expected to possess a molecular weight of ~6 kDa. Based on an hydroxyproline content of 10 mol%, there would be four to five hydroxyproline residues per protein molecule. If all hydroxyproline residues were glycosylated, this would mean that each hydroxyproline residue carried a polysaccharide chain containing approximately 1000 residues. Such a structure would be unprecedented. Fincher et al.[19] have reported sidechains of 100 residues for AGPs. Although there was no evidence for the existence in coffee bean cell walls of AGs which were not AGPs, it is possible that each of the AG fractions contained a mixture of a more protein enriched AGP fraction and a non-proteinated AG fraction which co-purified in the isolation procedures. The coffee AGPs represents an unusual group of AGPs. While the physiological function of AGPs has not been defined, they are not regarded as primarily a structural wall component. In coffee, they account for up to 25% of the primary cell wall and appear to fulfil just such a role.

There has been speculation that the AGs in the coffee bean cell wall are insoluble because they are covalently linked to an insoluble component of the wall such as the mannan or extensin. The purification of AG fractions that were essentially free of mannose suggested that the AGs and mannans in coffee are chemically separate polymers and are not covalently cross-linked, as it is unlikely the enzyme treatment would have able to cleave all the mannan if it had been covalently associated with AG.

Extensin is an hydroxyproline-rich protein but until now, hydroxyproline had not been reported in coffee cell walls. For the first time in this study, hydroxyproline has been identified in the cell-wall matrix. However, it appears that it is not part of extensin, nor is it the reason for the insolubility of the AGs within the wall. This conclusion is based on the fact that the AGPs in coffee can be solubilised by a variety of conditions, which include extraction in PAW, strong alkali, or by enzymatic degradation of the mannan–cellulose complex. The difference in solubility is likely to be related to where in the wall matrix a certain AG fraction is located in relation to the mannan–cellulose complex. An AGP which is situated on the outside of the fibrillar structure may be soluble in water or alkali, whereas AGPs located between fibrils may be inaccessible to solvation until the mannan–cellulose structure itself is solubilised.

This study reports the first structural evidence for the existence of a rhamnogalacturonan in the cell walls of the coffee bean. Previously it had been suggested that pectic polymers in coffee beans were contaminants left over from the pectic mucilage which envelops the endosperm.[1] This is unlikely, as such a contaminant would have been removed by one of several steps during the isolation of the 8 M KOH residue from which **AG 5** was derived. The rhamnogalacturonan had a MW average of 650 kDa and like the AG, was intimately associated with the wall matrix, being released only after dissolution of the bulk of the cellulose–mannan complex. In common with many pectins, it contained 2- and 2,4-linked rhamnosyl residues, the latter being the probable branchpoints in the galacturonan chain. Although most of the AG could be separated from the pectin fraction, a proportion of the AG (**AG 5 F2**) co-eluted with the rhamnogalacturonan from the anion exchanger, prompting the speculation that some AGPs may be linked to the pectin fraction.

Acknowledgements

The authors are grateful to Valerie Leloup and Charlotte Gancel for providing the coffee beans and for many helpful discussions. Thanks are due to Sylviane Metairon who conducted the GC–MS analyses, to Claudia Niemann for the HPSEC-Malls measurements and to Tony Bacic and Geoff Fincher for advice.

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